THE EFFECTS OF LEECH BEHAVIOR ON PENETRATION AND LOCALIZATION OF *APATEMON GRACILIS* (TREMATODA: STRIGEIDAE) CERCARIAE AND METACERCARIAE

James R. Palmieri[1] and Hugo A. James[2]

Abstract.— Metacercariae of *Apatemon gracilis* (Rudolphi 1819) (Trematoda: Strigeidae) were found to encyst primarily in the posterior half of their leech hosts (*Helobdella stagnalis, Placobdella parasitica, Erpobdella punctata*) in numbers of from 1 to 13 larvae per cyst. Presumably the posterior localization of these larvae in their leech hosts is determined by the host's behavior in response to cercarial penetration. It is possible also that leeches may become infected by ingesting snail tissues containing mature cercariae.

Several investigators have contributed to life-cycle studies and biology of *Apatemon gracilis* (Szidat 1928, 1929, 1931; Stunkard et al. 1941; Dubois and Rausch 1960; Iles 1960; Raishite 1967; and Palmieri 1973). Szidat (1929) found encysted metacercariae (tetracotyles) of *A. gracilis* in two leech species, *Herpobdella atomaria* and *Haemopis sanguisuga*. Szidat (1931) continued his investigation by studying cercarial penetration of leeches and subsequent development of cercariae to the tetracotyle stage. Most investigators since Szidat's (1929) report have agreed that leeches are the major second intermediate host in the life cycle of *A. gracilis*. Lists of leech species that harbor larvae of *Apatemon* may be found in McDonald (1969), Vojtek et al. (1967), and Dobrowolsky (1958). The various developmental stages of *Apatemon* have been well studied; however, there are apparently no reports concerning the behavior of leeches in response to invading cercariae. Although Erasmus (1962) observed that the number of metacercariae, typical of this genus, varied from 1 to 13 in a sample of 26 leeches (*Erpobdella octoculata*), he did not consider the localization of these larvae in the body regions of the leech hosts.

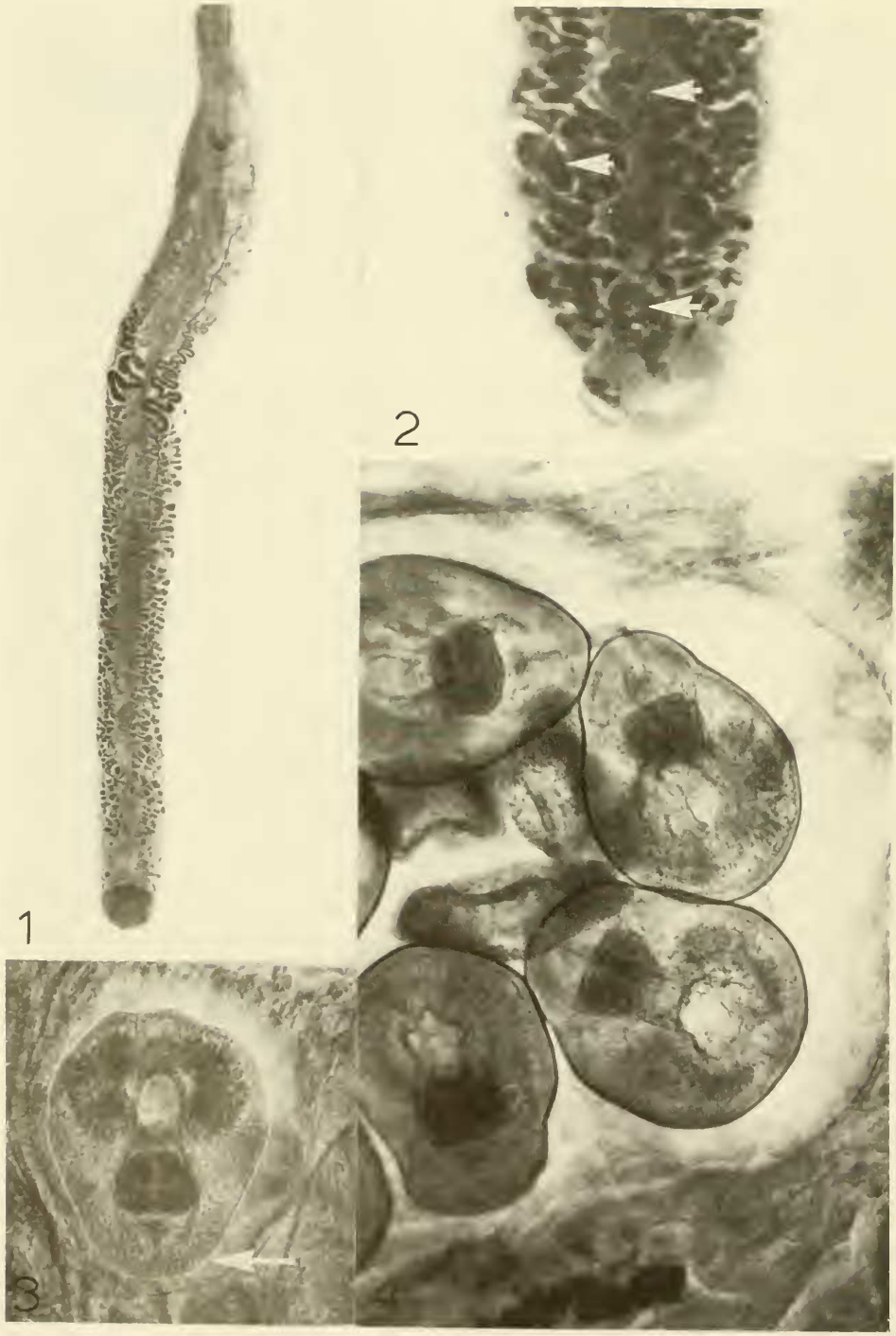
Materials and Methods

In this study, initiated during the early fall of 1967 and continued until spring of 1971, leeches (*Helobdella stagnalis, Placobdella parasitica,* and *Erpobdella punctata*) were collected by hand from a small pond at Beardsley Park, Bridgeport, Connecticut. Newly collected leeches and snails were separated and maintained in aquaria. Leeches to be examined for metacercarial cysts of *A. gracilis* were sandwiched between two glass slides which were squeezed lightly and the resulting preparation studied with the aid of a dissecting microscope. Tetracotyles lying close to the body surface of the leech appear as small, transparent globes. In uncleared leeches cercariae which have just penetrated the integument are difficult to see; therefore, leeches presumed to be uninfected were separated from infected specimens and reexamined in 30 to 60 days for the presence of metacercariae.

Snails *Physa gyrina* and *P. ancellaria*), segregated by species, were placed in four-inch culture dishes (five per dish) half filled with water; subsequently, those snails shedding furcocercous cercariae were put into aquaria containing leeches. Nonshedding snails were either crushed and examined for developing sporocysts or placed into holding tanks as a food source for experimental leeches. Snails were permitted to feed freely on algae and uncooked lettuce and were considered free of *A. gracilis* if cercariae were not shed within 150 days.

Tetracotyles of *A. gracilis* were easily teased from tissues; those to be fixed were either cold-relaxed or slightly flattened under coverslip pressure. Leeches for wholemounts were fixed, stained, and mounted according to methods of Palmieri et al. (1973). Leeches for experimental infection were placed in culture dishes containing snails shedding cercariae. The behavior of leeches in response to cercarial penetration was observed with the aid of a dissecting microscope.

[1]Department of Zoology, Brigham Young University, Provo, Utah 84602.
[2]Biology Department, University of Bridgeport, Bridgeport, Connecticut 06602.



Figs. 1-2. 1, Wholemount of *Erpobdella punctata* showing distribution of metacercariae along the length of its body; 2, Wholemount of the posterior region of an infected *Erpobdella punctata* showing great numbers of both single and multiple metacercariae per cyst.

Figs. 3-4. 3, Typical metacercariae of *Apatemon gracilis* encysted in leech tissue; 4, A cyst of *Apatemon gracilis*, located within the posterior region of the leech host, containing several metacercariae.

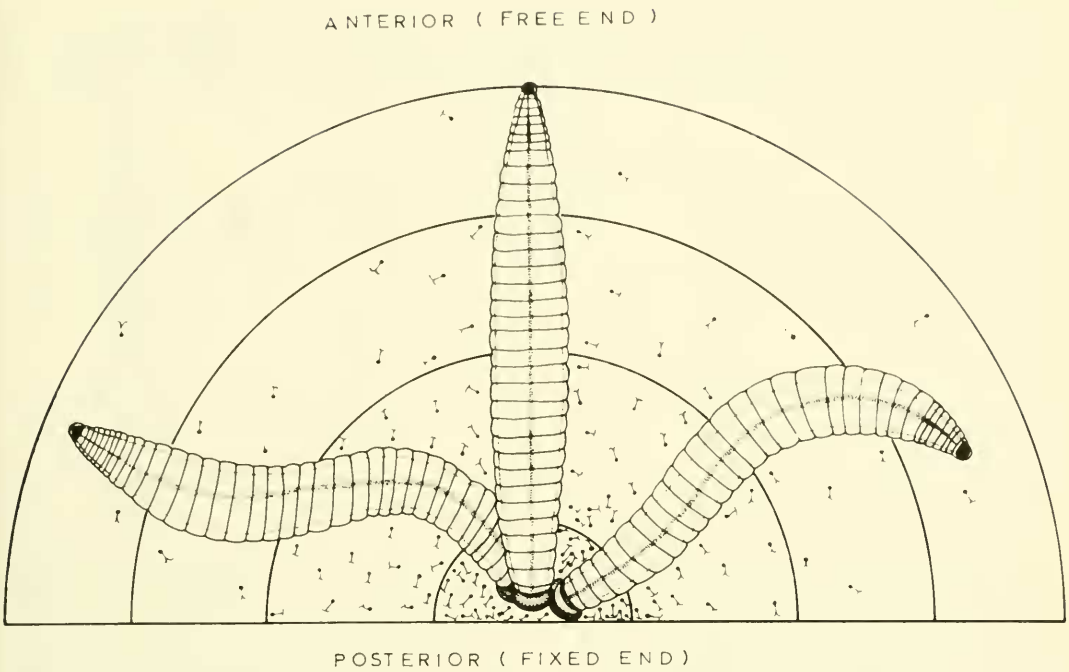


Fig. 5. Illustration to demonstrate the behavior of a leech upon penetration of the cercariae showing waving motion of the anterior (free end) and pivot attachment point of the posterior (fixed end); dots represent approximation/cercarial density.

Results and Discussion

In comparison with the behavior of leeches observed in nature and in aquaria without cercariae, those placed in culture dishes with over 500 cercariae of *A. gracilis* from a shedding snail increased their body undulations and secreted additional mucus. The greater number of cercariae attached to and penetrated the posterior half of the leech's body (Fig. 5). In 17 of 19 infected leeches examined, 50 percent or more of the metacercariae localized in the hind body, with the greatest number in the vicinity of the posterior sucker (Figs. 1, 2, 5).

Observation of leech behavior just prior to and during the course of cercarial penetration has provided an explanation for the consistent posterior localization of *A. gracilis* metacercariae in the leech host. A leech, upon being penetrated by a large number of cercariae, swings its anterior (free end) in an axis of 140° to 180° while pivoting on its posterior sucker (fixed end) (Fig. 5). The posterior end usually remained attached and showed little movement while the anterior end oscillated at a more rapid rate. It seems that the movements of the leech, in response to its parasites, determine the posterior localization of these parasites within their host. It is more likely that swimming cercariae can attach to the more stationary posterior region than to the faster moving anterior end. Cercariae, once attached to the host, lose their tails and penetrate the leech integument, seldom migrating from their point of entrance.

It might also be possible that infections of *A. gracilis* are acquired by leeches through ingestion of infective cercariae in snail tissues. Four *Erpobdella punctata* were discovered in the hepatopancreas and gonad of one infected snail where they had apparently been feeding. These leeches, when removed from the snail and examined under a dissecting microscope, had cercariae moving in a posterior direction in the leech's gut. Whether they matured into viable tetracotyles was not determined.

Naturally infected leeches described in this study contained more metacercariae than did those reported by Erasmus (1962). In one naturally infected 57 mm leech (*E. punctata*), 296 metacercariae,

Table 1: Metacercarial cyst distribution in leech hosts.

Leech[1]	Leech Length mm.	Cysts per leech	Cysts in posterior half of body	Cysts in anterior half of body	Percent cysts in anterior and posterior regions of leeches
H	8	10	7	3	30/70
P	16	1	1	0	0/100
E	57	296	203	93	31/69
E	13	46	36	10	22/78
E	26	228	120	108	47/53
E	32	54	43	11	20/80
E	28	8	26	2	25/75
E	10	3	3	0	0/100
E	24	42	39	3	7/93
E	7	12	9	3	25/75
E	14	40	20	20	50/50
E	24	25	15	10	40/60
E	28	128	79	49	38/62
E	31	102	73	29	28/72
E	34	197	133	64	33/67
E	9	1	0	1	100/0
E	12	1	0	1	100/0
E	11	14	12	2	14/86
E	24	21	13	8	38/62

in varying stages of maturation, were found (Table 1). In this investigation clumps of metacercariae (as many as 13) were found in cavities created by the known lytic activity of the metacercariae (Fig. 4) upon the leech botryoidal tissue, although a given cyst containing but a single metacercariae (Fig. 3) is more common. The larger leeches typically contained a greater number of metacercariae (Table 1), which probably reflects a continuing cercarial infection of the host.

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